



RECORD OF *DOWNSIOMYIA ALBOLATERALIS* (THEOBALD) (DIPTERA: CULICIDAE) FROM THE HIMALAYAN PLAINS OF WEST BENGAL

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ABSTRACT

The present study was carried out with the aim to characterize a mosquito species that was documented near about a century ago from Darjeeling district of northern West Bengal, India. Mosquito larvae were collected from the bamboo stumps or holes of sub-Himalayan plains of Darjeeling district, West Bengal, India. Collected immatures were reared upto adults maintaining standard protocol. Larva and adult specimens were investigated for morphological identifications upto generic and species level. In the present study, the Niveus species *Downsiomyia albolateralis* (Theobald, 1908) is a newer addition to the mosquito fauna in the region and it will update the mosquito checklist for the knowledge of vector and non-vector species.

Key words: *Downsiomyia albolateralis*, Culicidae, new record, morphology, mosquito, Niveus subgroup, rediscovery, taxonomy, vector, Darjeeling, West Bengal

The Niveus subgroup members are identified as important vectors of dengue virus and the human lymphatic filariasis parasite, *Wuchereria bancrofti* in the countries of South and Southeast Asia (Shriram et al., 2014; Choochote, 2001). A recent finding has estimated that 30 species of the Genus *Downsiomyia* Vargas, 1950 including the Niveus subgroup are found throughout the Oriental and adjoining Palearctic and Australian regions (Harbach, 2022). In India, according to Tyagi et al. (2015), there are currently six species of the genus, namely- *Do. albolateralis* (Theobald, 1908), *Do. albonivea* (Barraud, 1934), *Do. mohani* (Knight, 1969), *Do. nivea* (Ludlow, 1903), *Do. niveoides* (Barraud, 1934) and *Do. novonivea* (Barraud, 1934). The type species of Niveus subgroup *Downsiomyia nivea* Ludlow, 1903 is the only established vector of Diurnally subperiodic *Wuchereria bancrofti* (DspWB) form of filariasis restricted in the Nicobar district of Andaman and Nicobar Islands, India (Shriram et al., 2014).

Downsiomyia albolateralis Theobald, 1908 was first recorded from Assam hills, India by Theobald in the year 1908 (Theobald, 1908). Later the species was recorded from two northern districts of sub-Himalayan West Bengal, India, namely- Darjeeling in 1922, 1928 and Jalpaiguri in 1923 (Barraud, 1934). The species was also recorded from several other North-eastern (Mahanta et al., 2010; Rajput and Singh, 1992; Rajput and Kulkarni,

1991), North-western Himalayan states (Debi and Jauhari, 2007) and the Eastern and Western Ghat ranges of South India (Selvan et al., 2016; Munirathinam et al., 2014). Believably, *Do. albolateralis* is native to South and Southeast Asian countries e.g., India (Barraud, 1934), Pakistan (Jabeen et al., 2019), China (Maquart et al., 2021), Nepal (Darsie Jr. et al., 1993), Bangladesh (Irish et al., 2016), Sri Lanka (Gunathilaka, 2018), Myanmar, Cambodia (Maquart et al., 2021), Thailand (Choochote, 2001), Malaysia (Colless, 1958), Singapore (Colless, 1958) and Papua New Guinea (Maquart et al., 2021). In the current investigation by Zoological Survey of India, *Do. albolateralis* has been recorded from the western Himalayan hills although the eastern Himalaya, including sub-Himalayan plains of West Bengal, still awaits documentation about its occurrence (Banerjee et al., 2018). Recently 50 different mosquito species, without *Do. albolateralis*, belonging to 8 different genera have been documented and compiled in a study from this northern part of West Bengal (Saha and Saha, 2021) justifying its absence from the entire West Bengal for nearly a century, i.e., precisely after 1920s.

This species commonly breeds and lays their eggs in the forest tree holes, bamboo holes or stumps, leaf axils and rock holes at an elevation of about 300–3000 meters (Munirathinam et al., 2014; Mahanta et al., 2010; Debi and Jauhari, 2007; Rajput and Singh, 1992; Rajput and Kulkarni, 1991). Though Niveus

subgroup members are generally regarded as zoophilic but this species is a day biter, bites both humans and cattle (Rajput and Kulkarni, 1991; Choochote, 2001). Laboratory investigations in Thailand reported that, *Do. albolateralis* is 9.43% susceptible to bancroftian filariasis and 100% susceptible to DEN-2 virus as a competent vector of these pathogens (Choochote, 2001). Medical importance of the Genus *Downsiomyia*, especially the species *Do. albolateralis* has not been still widely studied likely due to its evasive behaviour but also because its identification is difficult with the existing keys and poor literature descriptions.

MATERIALS AND METHODS

Field surveys were conducted in the sub-Himalayan plains of Darjeeling district, the northern most part of West Bengal state, India (26°26'50"-26°58'00"N, 88°06'13"-88°31'03" E). The study area receives annual mean rainfall of 225 to 275 cm between June to September with an average temperature extension of 13.8 to 28.6°C giving the zone a tropical or sub-tropical climatic conditions. The study area also shares international borders with neighbouring nations, like Nepal in the west and Bangladesh in the south-east. The meteorological conditions of the region and also its great vegetation with diverse habitats render the area highly vulnerable to mosquito breeding. Immature stages of mosquitoes were collected from the natural breeding habitats of mosquitoes, like- bamboo holes or stumps and sticks. Mosquito sampling was conducted in five locations: Naxalbari, Matigara, Khoribari, Phansidewa and Sukna, in the plains of Darjeeling district between September and November, 2022. All the immatures were collected in 500 ml plastic containers, brought to the laboratory and reared upto adults (F_0) in separate rearing cages for each sampling site. Rearing was done maintaining $25 \pm 2^\circ\text{C}$, and 70-80% RH according to the standard protocol as described by Clemons et al. (2010). Fourth-instar larva and adult mosquito specimens were thoroughly investigated under a stereoscopic binocular microscope, Magnus MS-24 for morphological identifications. Standard identification keys were used for systematic identification of the specimens up to genus (Tyagi et al., 2015; Rueda, 2004) and species level (Colless 1958; Barraud 1934; Theobald 1908). Genitalia of the morphologically identified adult male mosquito specimens were dissected out and slide mounted according to the methodology described by Anuradha et al. (2022) and Rattanarithikul (1982). Male genitalia were investigated under Olympus OIC (708139) microscope.

RESULTS AND DISCUSSION

A total of 77 individuals were identified as *Do. albolateralis* among all the collected samples from the sub-Himalayan plains of Darjeeling district (Table 1); of the five sampling sites, four sites were found to be positive breeding habitats (PBH) for this species with other mosquitoes in mixed population; 43 were male and 34 were female. Majority (45 out of 77) of individuals were found from the bamboo stumps of Matigara. 19 individuals were yielded from Sukna, 12 individuals from Naxalbari area and a single individual was found from Khoribari area. Phansidewa area was found to be a negative breeding habitat (NBH) for the species in the present study (Table 1).

***Downsiomyia albolateralis* (Theobald, 1908)** (Fig. 1, 2)

Taxonomic account: Superfamily- Culicoidea Malloch, 1917; Family- Culicidae Meigen, 1818; Subfamily- Culicinae Meigen, 1818; Tribe- Aedini Neveu-Lemaire, 1902; Genus- *Downsiomyia* Vargas, 1950. Recent phylogenetic investigations on the classification of the tribe Aedini postulated that the species of Niveus Subgroup of the subgenus *Finlaya* Theobald, 1903 of the genus *Aedes* Meigen, 1818 has been instated in the genus *Downsiomyia* Vargas, 1950 (Natarajan, 2022; Reinert and Harbach, 2006). Therefore, *Aedes (Finlaya) albolateralis* has been reclassified and is currently known as *Downsiomyia albolateralis*.

Diagnosis: *Downsiomyia albolateralis* is a species-member of Niveus Subgroup, mainly distinguished by the male genitalia as females are sometimes undifferentiated. Like other species of the subgroup, this species has flat silvery-white scales on head and scutellum. A single, large, distinct silvery-white patch on front of mesonotum. Thorax with a broad silvery-white patch on each side in front. Legs dark, basal $\frac{2}{3}$ of hind-femur white. Wings dark, almost 3 mm. In male, gonocoxite having leaf-like long lanoeolate scales at its base. In larva, comb scales 8-11 and siphon slightly wider in middle than the either ends.

Description: Female (Fig. 1A-J). Medium-size mosquitoes. Head. Proboscis (P) slender, straight and black; maxillary palp (MPlp) short and black with few white scales at apex (Fig. 1B); with a thin border of silvery scales around eye-margins, a wider white patch of flat scales in the middle on front of vertex on head (H) (Fig. 1C); Thorax. Single large roundish

Table 1. Sampling sites, and other details of *D. albolateralis* (September-November, 2022)

Sampling sites	Representative coordinates	Habitat type	<i>Do. albolateralis</i> (n)	Sex	Population shared with
Naxalbari	26°43'4.3968"N 88°20'38.5764"E	Bamboo stumps (peri-domestic)	12	M-11 F-1	<i>Aedes albopictus</i> , <i>Culex quinquefasciatus</i> , <i>Cx. fuscocephalus</i> <i>Toxorhynchites</i> sp.
Matigara	26°44'10.9068"N 88°22'52.6332"E	Bamboo stumps (Tea Garden)	45	M-19 F- 26	<i>Ae. albopictus</i> , <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>
Khoribari	26°32'18.042"N 88°7'14.484"E	Bamboo stumps (Forest patch)	1	M-0 F- 1	<i>Ae. albopictus</i> , <i>Armigeres</i> sp.,
Phansidewa	26°40'24.4632"N 88°22'43.3884"E	Bamboo stumps (peri-domestic)	Nil	Nil	<i>Ae. albopictus</i> <i>Cx. quinquefasciatus</i> <i>Armigeres</i> sp.
Sukna	26°47'22.0416"N 88°21'55.5228"E	Bamboo stumps (Forested area)	19	M-13 F-6	<i>Ae. albopictus</i> <i>Cx. quinquefasciatus</i>

**Ae.* = *Aedes*; *Cx.* = *Culex*; *Do.* = *Downsiomyia*; F = Female, M = Males

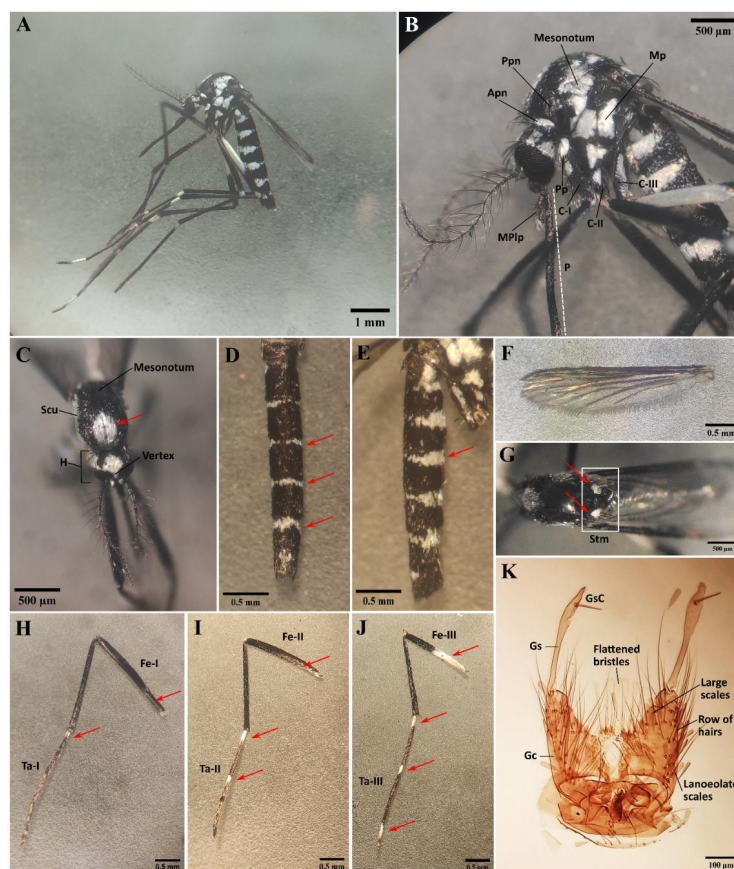


Fig. 1. *Downsiomyia albolateralis*- Adult female: A, Whole mosquito specimen; B, Lateral view of thorax; C, Dorsal view of head and scutum; D, Dorsal view of abdomen; E, Lateral view of abdomen; F, Wings; G, Dorsal view of mesonotum and scutellum; H, Fore leg; I, Mid leg; J, Hind leg; K, Dorsal view of male genitalia. Apn, antepronotum; C, coxa; Fe, femur; Gc, Gonocoxite; Gs, Gonostylus; GsC, Gonostylar claw; H, head; Mp, mesepimeron; MPlp, Maxillary palp; P, proboscis; Pp, propleura; Ppn, postpronotum; Scu, scutum; Stm, scutellum; Ta-tarsi (red coloured arrow showing pale/white streak, white band or white scale patch on different structures of the figures accordingly).

silvery-white patch of scales in front of mesonotum (Fig. 1C, showed by red arrow); Mesonotum having a large silvery-white scaling on its either side, forming the large lateral patches (Fig. 1B); Mesepimeron (Mp) largely covered by an elongated patch of silvery-white scales, another one silvery patch is in front of it and the other one below (Fig. 1B); Small silvery-patches on coxa (C), propleura (Pp), post-pronotum (Ppn) and ante-pronotum (Apn) (Fig. 1B); Scutellum (Stm) trilobed, lobes of either side have white flat scales (Fig. 1G); Abdomen. Abdomen dark-black, basal bands of large white scales form wedge-shaped markings on lateral side but not extended to dorsum (Fig. 1D); basal markings form narrow bands on Vth to VIIth abdominal segments, band on VIIIth segment quite large (Fig. 1E, showed by red arrow); Legs. Posterior $\frac{2}{3}$ or more of hind-femur (F-III) is entirely white, hind-tarsomeres (Ta-III) with 3 white bands (Fig. 1J); Mid-femur (F-II) anteriorly black and basal $\frac{1}{3}$ of it more extensively pale, some silvery scaling on anterior surface, tibia black and tarsi (Ta-II) paler with 2 white bands (Fig. 1I); Fore-femur (F-I) anteriorly black and basal $\frac{1}{3}$ of it has a pale streak beneath, tibia and tarsi (Ta-I) entirely black or brownish-black, paler at anterior end (Fig. 1H); Wings. Wings with dark scales and about 3 mm long (Fig. 1F).

Male (Fig. 1K). Males are little lighter than females or brownish. Smaller in size in comparison to females. Head. Head scales creamy or light brownish in colour; Maxillary palp black having a single white scale-band at anterior part near apex; palpi almost similar or a little shorter than proboscis in length. Antennal plumes dense and brown. Thorax. Anterior $\frac{2}{3}$ of mesonotum covered with white scales and other markings are very similar to those of ♀. No white scales above wing-

roots. Legs. Legs are similar to that of ♀. Abdomen. Abdomen- slender, ninth abdominal segment modified into hypopygial structure. Hypopygium (Fig. 1K). The gonocoxite is narrower and conical; A pair of submedian lobes placed together closely, each with 4 strong flattened bristles; the gonostylus (Gs) is almost equal to the length of its gonocoxite (Gc), long gonostylar claw (GsC) joints ventrally near the expanded apex of gonostylus; Gonocoxite having a group of large scales on ventral border, dorsal border with a row of hairs and leaf-like long lanceolate scales at its base (Fig. 1K).

Larva, 4th instar (Fig. 2). Number of hairs having few short, fine branches on thorax and abdomen; Lateral hairs of abdomen- large, 2-3 branched; Comb scales 8 to 11 in number, largely pointed, arranged in a single row (Fig. 2A, marked with white box); Sub-siphonal tuft with 6 branches (Fig. 2A); Siphon. Slightly wider in middle than the either ends, length >3x than that of its base (Fig. 2B); Pecten with 15 small teeth, each with one large and one or two small denticles, pecten covers $\frac{1}{2}$ of the tube from base, a hair-tuft with 3 branches at middle of the tube (Fig. 2B, marked with white box); Saddle almost encloses the anal segment (Fig. 2B).

Distribution in India: The species is distributed over all the seven states of North-eastern India, Garhwal region of Western Himalayas, Bastar district of Madhya Pradesh, Darjeeling district (Sukna and Kurseong) and Jalpaiguri dooars of northern West Bengal, Western Ghat hill ranges, and Nicobar district of Andaman and Nicobar Islands. But among the above-mentioned regions, *Downsiomyia albolateralis* has not been reported from Darjeeling and Jalpaiguri districts of the northern West Bengal. The species was lastly recorded

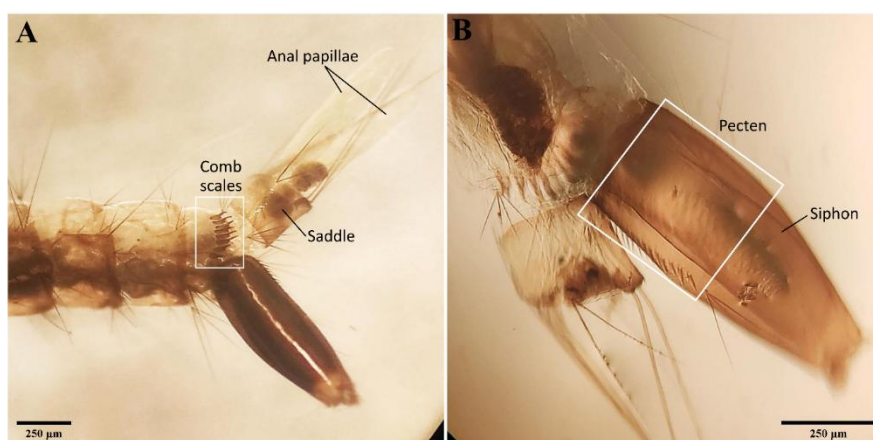


Fig. 2. *Downsiomyia albolateralis*. Fourth-instar larva: A, Posterior abdominal segments; B, Siphon (white-coloured boxes are used for denoting different minute structures on the figures).

from Sukna and Kurseong of Darjeeling and dooars region of Jalpaiguri district during 1920s, about a century ago.

Bionomics: Immature stages of *Do. albolateralis* were found from the bamboo stumps of PBHs in the study area. Adults were emerged in the laboratory after collection. Immatures of the species were co-existing with the other mosquito species, like- *Aedes aegypti* Linnaeus, 1762, *Ae. albopictus* Skuse, 1895, *Armigeres* sp. Theobald, 1901, *Culex fuscocephalus* Theobald, 1907, *Cx. quinquefasciatus* Say, 1823 and *Toxorhynchites* sp. Theobald, 1901 in the mixed population. Bionomics of the species is not known in greater details.

The description given here about *Do. albolateralis* is based on our present study over 34 adult females, 43 males with their male genital characters and a number of fourth-instar larval specimens collected from the sub-Himalayan plains of Darjeeling district. Majority of characters of the species *Do. albolateralis* described by Theobald (1908) and Barraud (1934) are found to be similar to that of our described specimens. The major characters include: in adult females, a wider white patch in the middle on front of vertex on head; a single large roundish silvery-white patch in front of mesonotum; posterior $\frac{2}{3}$ of hind-femur is entirely white; wings with dark scales. In the male genitalia, dorsal border of gonocoxite with a row of hairs and leaf-like long lanoeolate scales at its base. In the fourth instar larva, comb scales largely pointed, 8 to 11 in number, arranged in a single row; Pecten with 15 small teeth and wider in the middle region. *Do. albolateralis* females show slight differences from the nominotypical member of *niveus* subgroup, *Do. nivea* which possess a larger solid white area on front of mesonotum covering $\frac{2}{3}$ area of it and all scutellar scales are black. *Do. nivea* males have coxite devoid of scales at the base but have long hairs in that position. *Do. albolateralis* larva also differ from *Do. nivea* in comb scale number, siphon length and the number of pecten teeth. Other *Niveus* species namely- *Do. alboniveus*, *Do. niveoides* and *Do. novoniveus* are largely indistinguishable from *Do. nivea* adult but show distinguishable differences in adult female characters and larval structures of *Do. albolateralis*. Females of these species do not show any external differences from *Do. nivea* where the white spot on mesonotum is rather wider and complete. In *Do. albolateralis*, the white-spot is distinct, roundish and restricted only on front of mesonotum. Adults of this subgroup are almost identical and only can be distinguished by the male

genital structures. Presence of large leaf-like lanoeolate scales at the basal area of gonocoxite in males, is the distinguishing character of this species from other members of the subgroup.

However, there are few minor characters that differing from the literature descriptions (Theobald 1908; Barraud 1934; Colless 1958) in having distinct white colour bands on hind-tarsi (Ta-III) and mid-tarsi (Ta-II), a collection of few pale-white scales in postpronotum (Ppn) and few white scales at the apex of maxillary pulp (MPlp) in female and a single white scale-band at anterior part (near apex) of maxillary pulp in male. Although few minor characters differing from those described by Theobald (1908) and Barraud (1934), major characters of adult females, males (male genitalia) and fourth instar larva examined are seemed to be sufficient to recognise them as *Do. albolateralis* Theobald. Globally, there is no morphology-based identification key available that describes the present species. Thus, the species level identification is very difficult with the existing keys and inadequate literature descriptions. The only illustrated description was made by Barraud (1934) published under the world-famous book- 'The Fauna of British India including Ceylon and Burma' series where the species was reported from Sukna (1922) and Kurseong (1928) in Darjeeling district more than a century ago. The first record of mosquitoes from the sub-Himalayan foothills when published in 1970, it reported 18 culicine species but did not mention *Do. albolateralis* (Varma and Mahadevan, 1970). In later studies also, the species has not been reported from this region (Saha and Saha, 2021; Rudra and Mukhopadhyay, 2010; Aditya et al., 2006; Malakar et al., 1995). Now, reporting of the species in the present study simply depicts it re-occurrence after about 100 years from this region and the first record of the species- *Do. albolateralis* Theobald from the northern part of West Bengal after Indian independence in 1947. The present study cites and compiles all the possible available data and descriptions best known to date for the species.

The *Niveus* species *Do. albolateralis* was found to be a potential vector for NspWB and DENV in laboratory but their role as natural vector for these two diseases is still unknown in India (Choochote, 2001). One of its close relatives *Do. nivea* and some members of this group (also including *Do. albolateralis*) have been implicated in the transmission of DspWB among the Nicobarese of Andaman and Nicobar islands (Shriram et al., 2015) and NspWB in Thailand (Gould

et. al., 1982). Now *Do. albolateralis* is resurging in the sub-Himalayan Darjeeling district which is among the prevalently dengue endemic districts of northern West Bengal (Roy et al., 2022; Bharati et al., 2021; Taraphdar et al., 2010). The two adjacent districts, namely-Cooch-Bihar is endemic for nocturnally periodic *W. bancrofti* (NpWB) form of filariasis (Saha and Saha, 2021) and Jalpaiguri is another prevalently dengue endemic districts of northern West Bengal (Bharati et al., 2021). The species is found here in association with the established primary vectors of dengue namely- *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse and principal lymphatic filariasis vector, *Culex quinquefasciatus* Say in the mixed populations (Table 1). Therefore, occurrence of this Niveus species in the region that has a potentiality for acting as an important vector elsewhere, has made a serious concern in the context of public health implications of the area. It may be a possible vector for these diseases in changing environmental conditions of upcoming days in absence of primary vectors. Rapid transformation of peripheral forest areas and tea plantations into urban set-ups in this region has resulted in the deterioration of the natural landscape in and around these urban centers. In earlier studies over India, the species have reported from the rural, semi-forested, hilly area having certain elevations but in the present study they are yielded from the urbanised, peri-domestic settlements of plains also (Table 1). These ultimately results into indiscriminate exposure of large population towards these jungle mosquitoes and increases the chances of zoonosis. The species documentation is an essential component of biodiversity study and helps to design the conservation programme of genetic resources as well as vector and pest control measures. The study will be helpful for better understanding of the bionomics and illustrated descriptions with greater details which were not previously documented well enough for this species. It will also be an addition to the mosquito faunal diversity and update the mosquito checklist of this area.

ACKNOWLEDGEMENTS

The authors thank the authorities of University of North Bengal for un-interrupted power supply and high-speed internet facility for accessing research articles. The Head, Department of Zoology, University of North Bengal is acknowledged for extending laboratory facilities and funds from the departmental budget.

FINANCIAL SUPPORT

No financial support.

AUTHOR CONTRIBUTION STATEMENT

Ratnadeep Sarkar. Roles. Data curation, Formal analysis, Investigation, Methodology, Resources, Writing- original draft and Writing- review and editing; Abhirup Saha. Roles. Data curation, Formal analysis, Investigation, Methodology, Resources and Writing- review and editing; Subhajit Das. Roles. Resources, Software and Visualization; Prapti Das. Roles. Formal analysis, Investigation and Writing- review and editing; Dhiraj Saha. Roles. Conceptualization, Supervision, Validation and Writing- review and editing

CONFLICT OF INTEREST

No conflict of interest.

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(Manuscript Received: August, 2023; Revised: December, 2023;

Accepted: December, 2023; Online Published: January. 2024)

Online First in www.entosocindia.org and indianentomology.org Ref. No. e23556